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Thyroid Morphology and Wet Organ Weight Changes in Rats Exposed to Different Low Intensity 0.5 Hz Magnetic Fields and Pre-Experimental Caging Conditions

by

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ABSTRACT. — In eight separate experiments, 48 adult female rats were caged in groups of 3 or 4 individuals under normal lighting conditions for 120-140 days. They were then exposed in pairs to one of 6 different variation intensities (0.001 to 10 G, i.e., 10^{-7} T to 10^{-3} T) of a 0.5 Hz rotating magnetic field (RMF) or to one of 3 sham field positions for 10 days while under constant 45 lux illumination. Post-mortem examination and histological analyses demonstrated that RMF-exposed rats show no significant changes in thyroid follicle numbers, perifollicular mast cell numbers, wet adrenal and pituitary weights, body weight changes, or water consumption either between intensity groups or relative to sham field controls. Significant interactions existed for thyroid and spleen weights between exposure intensities and pre-exposure caging conditions for the 10^{-5} T to 10^{-6} T groups; the effect was not linearly related with field intensity. Rats that had been maintained in the 4/cage situation showed significantly lighter body weights and more thyroid follicles; the significant alterations in thyroid, adrenal and spleen weights were related to body weight.

INTRODUCTION

The thyroid has been implicated strongly in the major response profile associated with the presence of ELF (extremely low frequency) electromagnetic fields. Chickens exposed before birth to ELF pulsed fields displayed changes in thyroid follicle numbers (Neaga and Lazan, 1972) while rats exposed prenatally to 0.5 Hz rotating magnetic fields have displayed adult behaviors suggestive of thyroidal involvement (Persinger, 1969: Persinger and Foster, 1970; Persinger and Pear, 1972). Correlational data have indicated that thyroid activity (as measured by ^{131}I uptake) can be associated with labile weather conditions (Frey and Goeke, 1969) and research by König (1974), Ludwig (1974) and Lotmar and Ranscht-Froemsdorff (1968) have demonstrated the presence of ELF fields (pulses and waves) in the vicinity of such meteorological perturbations. Adult rats exposed for several days to 0.5 Hz magnetic fields have displayed thyroid-like symptoms such as ambulation changes (Persinger et al., 1972), increased water consumption, testicular and thyroidal weight changes but no changes in wet adrenal or pituitary weights (Persinger, Glavin and Ossenkopp, 1972). However, these effects were associated with field variation intensities between 10^{-3} T and 10^{-4} T, several orders of magnitude above normal biometeorological-geophysical source values. A review of the above research has been prepared by Persinger, Lafrenière and Ossenkopp (1974).

Changes in wet thyroid weights are subject to extirpation error and are non-specific.

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Consequently, we decided to determine if any significant morphological (histological) modifications were associated with long term (10 days) exposures to 0.5 Hz rotating magnetic fields (RMFs). Thyroid follicle numbers and perifollicular mast cell numbers were selected as measures since the former reflects changes in gross thyroid demands and the latter are sensitive to plasma TSH (thyrotropin) concentrations (Ericson, Hakanson, Melander, Owman, and Sundler, 1973); perifollicular mast cells are clear histological units and appear to be a critical sequence in thyroid activation in the rat (Nunez and Gershon, 1973). In order to give any changes possible practical biometeorological relevance, a number of variation intensities were selected ranging from 10^{-3} T (previously associated with significant wet weight tissue changes) to 10^{-7} T(which approaches meteorogenic values). A final non-field variable: pre-exposure home cage subject density was selected as a magnitude comparator for any possible morphological changes associated with the ELF magnetic fields.

METHOD

SUBJECTS. — Forty-eight, 180 to 200 day old naive Wistar albino female rats were used as subjects; they had been obtained from Bio Breeding Laboratories of Canada, Ottawa, at 60 days of age.

APPARATUS. — The 0.5 Hz RMF (rotating magnetic field) was produced by permanent horseshoe magnets rotating about their major axes at 30 rot/min by an alectric motor. More detailed information on the construction of the apparatus has been reported elsewhere (Persinger, Lafrenière and Ossenkopp, 1974). Field variation intensities were manipulated by either: (1) systematically varying the distance of the exposure areas from the magnets (i.e., positions 1, 2 and 3), or (2) using either a "high" or "low" intensity set of magnets. With the "high" intensity set of magnets, exposure variation intensities at the 3 exposure positions were 10, 1 and 0.05 G, as measured by a RFL Industries Model No 750 Gaussmeter; with the "low" intensity magnets attached, exposure intensities were 0.1, 0.01, and 0.001 G in the same 3 positions (the lower intensity variations were confirmed with a McPhar magnetometer). The intensity overlap was intentional in order to control for possible differences between experiment sequences. The sham-conditions were created by removing the magnets and by placing subjects in the same 3 positions; previous measurements (Persinger et. al., 1974) have shown no differences in vibration or noise level between the magnet on or off conditions. Background *static* intensity (the geomagnetic-cultural field) in the experimental area was $80{,}000 \pm 500\gamma$ (the vertical component), as measured by a McPhar magnetometer.

PROCEDURE. — Between the arrival time from the breeder laboratories and the beginning of the experiment, the subjects were kept in either groups of three or four in standard 40.5 cm X 24 cm X 18 cm (deep) steel "home" cages. Care was taken during this period that these subjects were maintained on food and water ad libitum at all times. During the pre-experimental period, the following colony room conditions were maintained: homeostat controlled temperature 22 ± 1°C and a 12:12h L:D schedule.

Groups of six subjects were exposed for 10 days to either: (1) the "high" intensity magnetic variations, (RMF-H) (2) the "low" intensity variations (RMF-L), or (3) the sham-field conditions (S), according to the following experimental sequence: S, RMF-H, RMF-H, S, RMF-L, RMF-L, S and RMF-H. Selection of subjects for each group was counterbalanced for home cage condition (number of animals/cage) and rack position. During each experimental sequence, two subjects from different home cages were placed in one of three 28 cm X 18 X 12 cm (deep) plastic cages and placed in one of the three experimental positions; the major concentration of applied fields was

oriented through the plastic portion of the cages. The cages were covered with wire tops and surrounded by white poster-board such that light entered only from the overhead sources. Pathogen-free ground corn cob was used as absorbent material; the absorbent was always changed on the third and sixth days of the experiments. During these times, the subjects were removed from the experimental conditions for about 5 minutes.

Subjects exposed to the sham-field conditions were placed in the same type cages and in the same positions used in the RMF-L and RMF-H sequences, except the magnets had been removed from the apparatus. The magnetometer measured no detectable 0.5 Hz variation within 10^{-5} G (i.e., 1 γ) within the sham-field areas.

The plastic cages, water bottles, and nozzles were washed with soap and rinsed before each experimental sequence. Ambient temperature (measured around the outside of each cage), carefully controlled by a homeostat for all experimental areas during all sequences, was 22.5 $\pm$ 0.03°C. Spontaneous auditory stimuli above the background masking noise of the motor (70 $\pm$ 2 db) was minimal, except during the two periods of cage cleaning. Indirect lighting was continuous from fluorescent lamps 2 m overhead and measured 45 $\pm$ 5 lux in each cage. Purina rat chow and tap water were available ad libitum; water consumption for pairs of rats in each of the cages was measured daily after which fresh tap water was given.

On the last days, a subject was removed from its cage, weighed, brought to the analysis lab and decapitated; time of removal to decapitation averaged 2.5 min. Thyroids, hypophyses, spleens and adrenals were quickly extirpated and weighed following removal of adipose/connective tissue. The animal decapitation order was the same for all sequences; the time between the killing of the first animal and the second animal from any given cage was always between 35 to 40 minutes.

Vaginal smears taken at death were stained with thionin in order to determine oestrus profiles. The thyroids were fixed in phosphate-buffered calcium formalin, stored for 16 to 20 h at 4°C and later paraffin embedded for histological examination. Care was taken to prevent differential dehydration between tissue batches. Thyroids were sectioned at 6 μm; different slides for each animal were stained with either hemotoxylin and eosin for follicle counting or with Lillie's Thionin Method (Humason, 1972) for mast cells. The number of follicles per unit area for each thyroid, derived by averaging the results of two non-adjacent slides from the central volume of the tissue, were measured with a Bausch and Lomb Tri-simplex by G.F.L. Mast cell numbers for each subject's thyroid were determined by N.J.C. and M.A.P.; four entire sections (2/lobe) for each rat were traversed completely with a grid eye piece at 400 X. Only mast cells between follicles were counted; those associated with peripheral connective tissue were not. All data were analyzed by IBM 360-40 computer using SPSS programs for analyses of variance and covariance as well as means and variance measures.

RESULTS

The means and standard deviations for absolute wet weights for pituitaries, thyroids, adrenals and spleens as well as thyroid follicle numbers, perifollicular mast cell numbers, percent body weight change during exposure, and terminal body weight for rats that had been exposed to the six variation intensities (in Tesla) and three sham field positions (S1, 2, 3) are presented in Table 1. The means and standard deviations for these measures according to pre-exposure conditions of 3 rats/home cage and 4 rats/home cage are presented in Table 2.

Two way analyses of variance (6 RMF + 3 sham field = 9 field exposure conditions by 2 pre-exposure cage conditions), performed on the above measures as well as relative tissue weights in order to detect possible non-linear body weight effects, demonstrated no statistically significant differences for any of the measures between the nine exposure conditions. Thyroid weights for RMF rats only (n = 30) displayed a cubic term

($F = 4.29$, $df = 1, 24$; $P < .05$) with field intensity according to polynominal analyses. Other measures demonstrated neither significant linear, quadratic or cubic terms with intensity.

Table 1. Means ($\bar{x}$) and standard deviations ($\pm$) for tissue measures for 48 rats exposed to different 0.5 Hz magnetic field variation intensities or sham field positions.

		Variation Intensities						Sham Positions		
		10^{-3}T (1) (n=6)	10^{-4}T (2) (n=6)	10^{-5}T (1) (n=4)	$5x10^{-6}$T (3) (n=6)	10^{-6}T (2) (n=4)	10^{-7}T (3) (n=4)	1 (n=6)	2 (n=6)	3 (n=6)
Pituitaries	$\bar{X}$	20.6	21.3	19.7	20.1	19.3	18.8	18.6	19.2	20.9
(mg)	SD	4.5	4.5	4.5	2.2	2.3	1.7	3.0	5.6	3.3
Thyroid	$\bar{X}$	19.9	15.7	18.9	19.3	20.0	17.8	17.0	19.7	18.1
(mg)	SD	3.0	2.8	5.9	4.1	6.2	4.6	2.2	4.6	3.1
Adrenals	$\bar{X}$	52.2	55.6	58.0	55.8	55.9	55.0	54.9	55.4	59.0
(mg)	SD	5.9	8.1	5.3	8.2	7.0	8.6	8.5	11.7	12.4
Spleen	$\bar{X}$	703.0	593.0	675.0	627.0	660.0	592.0	620.0	587.0	590.0
(mg)	SD	150.0	65.0	108.0	89.0	89.0	101.0	101.0	112.0	81.0
Thyroid Follicles	$\bar{X}$	94.8	95.3	100.4	100.4	109.0	100.2	100.2	98.0	98.1
(Number/mm$_2$)	SD	15.2	7.1	8.3	10.4	11.2	10.1	11.0	17.6	6.8
Thyroid Mast Cells	$\bar{X}$	40.3	43.3	49.0	49.0	41.3	43.5	40.8	41.7	38.2
(Number/mm$_2$)	SD	7.1	4.0	6.8	8.0	5.9	4.0	13.1	10.1	8.6
Body Weight	$\bar{X}$	337.0	332.0	344.0	319.0	330.0	338.0	354.0	341.0	339.0
(g)	SD	28.0	26.0	25.0	56.0	24.0	24.0	37.0	54.0	65.0
Body Weight Change	$\bar{X}$	+0.1	+0.9	-0.4	+1.2	+0.6	0.0	-0.7	-0.9	-0.2
(&)	SD	2.1	4.1	2.6	1.3	1.8	1.3	1.4	1.0	1.5

Rats that had been maintained before field exposure in pre-exposure conditions of 4 rats/cage displayed lighter absolute pituitary weights ($F = 4.77$; $df = 1,30$; $P < 0.05$), heavier relative adrenal weights ($F = 12.04$; $P < 0.01$) heavier relative spleen weights ($F = 14.42$; $P < 0.01$), lighter body weights ($F = 21.69$; $P < 0.001$) and more thyroid follicle numbers ($F = 26.72$; $P < 0.001$) relative to groups that had been maintained in the 3 rats/cage condition. All other measures were not statistically significant. However, when all measures (except body weight) were covaried for body weight before analyses of variance, only the thyroid follicle numbers remained statistically significant ($F = 7.91$; $df = 1,29$; $P < 0.01$) between these groups. Body weight significantly ($F = 15.80$; $df = 1,29$; $P < 0.001$) contributed to the numbers of thyroid follicles in all 48 rats.

Significant exposure intensity condition by pre-exposure cage density interactions existed for relative thyroid weights ($F = 3.33$; $df = 8,30$; $P < 0.01$), and relative spleen weights ($F = 3.30$; $df = 8,30$; $P < 0.01$). Thyroid follicle interactions ($F = 2.20$) approached significance ($P < 0.10$). Interactions were apparent also for absolute thyroid weights ($F = 2.55$; $df = 8,29$; $P < 0.05$) and absolute spleen weights ($F = 2.47$; $df = 8,29$; $P < 0.05$) when the analyses of variance was performed following analyses of covariance for body weight. These interactions were due to the greater increases in tissue weights and thyroid follicle numbers for the 4 rats/cage condition relative to the 3 rats/home cage condition at the 10^{-6} T to 10^{-5} T intensities only. No other interactions were significant.

Table 2. Means (x) and standard deviations (±) for tissue measures for 48 rats that had been exposed to one of two pre-exposure caging density conditions.

		Caging Condition	
		3 Rats/Cage (n = 24)	*4 Rats/Cage* (n = 24)
Pituitary (mg)	$\bar{x}$	21.3*	18.5
	SD	5.7	2.0
Thyroids (mg)	$\bar{x}$	18.4	18.5
	SD	3.6	4.5
Adrenals (mg)	$\bar{x}$	56.1	55.3
	SD	8.5	8.4
Spleen (mg)	$\bar{x}$	614.0	637.0
	SD	109.0	99.0
Thyroid Follicles (Number/mm²)	$\bar{x}$	89.7*	110.0
	SD	9.5	11.5
Thyroid Mast Cells (Number/mm²)	$\bar{x}$	39.9	45.1
	SD	9.0	6.5
Body Weight (g)	$\bar{x}$	355.3*	319.1
	SD	34.9	35.7
Body Weight Change (%)	$\bar{x}$	0.0	0.2
	SD	1.9	2.4

* $P < 0.05$

The actual exposure position in the experimental apparatus, regardless of the magnetic field intensity, is indicated by the numbers in parentheses adjacent to the field intensity values and the sham positions in Table 1. A one-way analyses of variance indicated no significant position effect for any of the measurements. There were also no significant kill order effects.

A 7 X 9 analyses of variance (combining sham field groups) with one factor repeated to determine differences in water consumption (μl water/g tissue weight) for pairs of animals exposed to the various experimental conditions showed significant differences over days ($F = 17.47$; $P < 0.001$), but no significant differences between field intensities ($F = 1,29$). The mean water consumption for the 10, 1, 0.1, 0.05, 0.01, 0.001 gauss and sham-field conditions (average for 9 days) was 84, 94, 87, 65, 65, 52 and 68 μl/g, respectively. Mean water consumption for all groups combined for the nine days were 89, 73, 72, 74, 71, 69, 68 and 68 μl/g, respectively. Individual t-tests indicated that the significant differences were due primarily to the elevated water consumption by all groups on the first two days only; after this period mean daily water consumption did not deviate from "normal" fluctuations. Inspection of stained vaginal smears indicated that all but one of the 48 subjects were displaying early oestrus at the time of death; the aberrant rat displayed an abnormal kidney condition as determined by macroscopic examination.

DISCUSSION

Thyroid follicle numbers and perifollicular mast cell numbers did not respond significantly to the wide range of 0.5 Hz variation intensities employed in the present study.

The presence of significant field exposure by pre-exposure cage condition interactions for spleen and thyroid wet weights indicated that the field effect was dependent upon the animals' source. Animals that had been maintained in the higher density groups (4/cage) before the exposures displayed enhanced responses at the 10^{-5} T to 10^{-6} T variation levels relative to the lower density groups (3/cage).

The importance of pre-exposure environments of the subjects upon the direction and amplitude of the 0.5 Hz RMF effect has been discussed elsewhere (Persinger, 1974). In previous studies, female rats that had been maintained in automatically cleaned cages, characterized by periodic flushing and dampness, displayed 73.0 μg/g thyroid wet-eights when exposed to 10^{-4} T variation 0.5 Hz fields for 10 days relative to the sham field rats' 56.8 μg/g (Persinger, Lafrenière and Ossenkopp, 1974). Female rats with similar pre-exposure experience, exposed to either 10^{-3} T, 10^{-4} T, 10^{-6} T or 10^{-7} T RMFs or sham field conditions showed a peak and significant increase in thyroid follicle numbers relative to either sham field or higher intensity fields around 10^{-6} T variation (Persinger and Lafrenière, 1975). The other major parameters, e.g., ambient lighting during exposure, temperature, noise, were used also in the present study. We have concluded that whereas the RMF effect may be replicable within a given series of experiments involving animals of homogeneous housing experience alterations in pre-exposure conditions can strikingly alter the pattern of the effect.

It appears that the 10 day exposures to the RMF intensities used in this study do not induce Selye-like stress patterns since no significant changes were noted in wet adrenal or pituitary weights or in body weight changes during exposure relative to sham field controls. These patterns are commensurate with previous reports (Persinger, Glavin and Ossenkopp, 1972). Like previous studies, the higher intensity (10^{-4} T and 10^{-3} T) variation rats drank more water than the controls, although these differences were not statistically significant either between intensities or relative to sham field references. In the early studies, male rats housed in larger groups during exposure under more intense (500 lux) lighting, factors which influence water consumption, were studied.

On the other hand, the pre-exposure caging conditions were significantly more effective upon thyroid morphology and tissue weights. Whereas no significant alterations were noted in MC numbers, rats that had been maintained in higher cage densities (4/cage) displayed 23% increases in thyroid follicle numbers relative to groups maintained in less dense conditions (3 rats/cage). The relative intensity of the caging effect can be seen in the significant 10% reduction in body weight for the high density groups, a factor which significantly influenced the wet weights of several tissues as determined by covariance.

REFERENCES

ERICSON, L. E., HAKANSON, R., MELANDER, A., and SUNDLER, R. (1972): TSH-induced release of 5-hydroxytryptamine and histamine from rat thyroid mast cells. Endocrinology, 90: 795-801.

FREY, K-W. and GOEKE, I. (1969): Wetter und Radiospeicherung der Schilddrüse. Ärztl. Forsch., 23: 373-375.

HUMASON, E. (1972): Animal Tissue Techniques. Freeman, San Francisco, p. 349.

KÖNIG, H. L. (1974): ELF and VLF signal properties: physical characteristics. In: ELF and VLF Electromagnetic Field Effects. M. A. Persinger (ed.) Plenum Press, New York, 9-34.

LOTMAR, R. and RANSCHT-FROEMSDORFF, W. R. (1968): Intensität der Gewebeatmung und Wetterfactoren: Korrelation des $Q0_2$ von Kaninchenhaut und Atmospherics. Z. angew. Bäder. u. Klimaheilk, 15: 1-10.

LUDWIG, H. W. (1974): Electric and Magnetic Field Strengths in the Open and in Shielded Rooms in the ELF- to LF Zone. In: ELF and VLF Electromagnetic Field Effects. M. A. Persinger (ed.), Plenum Press, New York, 35-80.

NEAGA, N. and LAZAR, M. (1972): Histological changes in the thyroids of chicks after treatment with a pulsing magnetic field. Stud. Cercet. Biol., Ser. Zool., 23: 233-237.

NUNEZ, W. and GERSHON, M. (1973): Species differences in mast cells of the thyroid gland. Endocrinology, 92: 152-159.

PERSINGER, M. A. (1969): Open field behavior in rats exposed prenatally to a low intensity low frequency, rotating magnetic field. Develop. Psychobiol., 2: 168-171.

PERSINGER, M. A. (1974): ELF electric and magnetic field effects: the patterns and the problems. In: ELF and VLF Electromagnetic Field Effects. M. A. Persinger (ed.), Plenum Press, New York, 275-310.

PERSINGER, M. A. and FOSTER, W. S. (1970): ELF rotating magnetic fields: prenatal exposure and adult behavior. Arch. Met. Geoph. Bioklim. B., 18: 363-369.

PERSINGER, M. A., GLAVIN, G. B. and OSSENKOPP, K-P., (1972): Physiological changes in adult rats exposed to an ELF rotating magnetic field. Int. J. Biometeor., 16, 163-172.

PERSINGER, M. A. and LAFRENIERE, G. F. (1975): Relative hypertrophy of rat thyroid following 10 day exposures to an ELF magnetic field: determining intensity thresholds. In: Biometeorology, Volume 6, H. W. Landsberg and S. W. Tromp (ed.). Supp. to Vol. 19, 1975, Int. J. Biometeor., Part I, 126-127.

PERSINGER, M. A., LAFRENIERE, G. F. and OSSENKOPP, K-P., (1974): Behavioural, physiological, and histological changes in rats exposed during various developmental stages to ELF magnetic fields. In: ELF and VLF Electromagnetic Field Effects. M. A. Persinger (ed.), Plenum Press, New York, 177-225.

PERSINGER, M. A. and PEAR, J. J. (1972): Prenatal exposure to an ELF-rotating magnetic field and subsequent increase in conditioned suppression. Develop. Psychobiol., 5: 269-274.

PERSINGER, M. A., PERSINGER, M., OSSENKOPP, K-P., and GLAVIN, G. B. (1972): Behavioral changes in adult rats exposed to ELF magnetic fields. Int. J. Biometeor., 16: 155-162.